American Journal of Medical Genetics 70:253–260 (1997)

Unexpected Familial Recurrence in Angelman Syndrome

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We report on two instances of familial recurrence of Angelman syndrome which, from pedigree analysis, appear incompatible with currently known mechanisms of inheritance of this disorder. In these two families, deletion-positive Angelman syndrome has recurred in cousins. Several established mechanisms for deletion-positive familial recurrence have been ruled out. In each family, molecular cytogenetic studies show typical chromosome 15 deletions, and DNA methylation analysis verifies the maternal origin of the deleted chromosomes in all four individuals. Since the mothers of the affected individuals in each family are not known to be related, these recurrences appear to be secondary to coincidental, de novo events. This conclusion is consistent with direct and indirect estimates of the population frequency of Angelman syndrome. Am. J. Med. Genet. 70:253-260, 1997.

KEY WORDS: Angelman syndrome; chromosome 15 deletion; recurrence; familial

INTRODUCTION

Angelman syndrome (AS) [Angelman, 1965] is a neurogenetic disorder with a specific phenotype [Williams and Frias, 1982; Robb et al., 1989; Williams et al., 1995a] that is caused by the functional absence of the maternal copy of a segment of chromosome 15, which can result from several mechanisms. About 70–80% of individuals clinically diagnosed with AS have a demonstrable interstitial deletion within the maternally derived chromosome 15q11-13 segment [Knoll et al., 1989; Magenis et al., 1990; Beuten et al., 1993; Saitoh et al., 1994; Zackowski et al., 1993]. The significance of the deletion's maternal origin in this disorder is attributed to imprinting effects on this portion of chromosome 15, wherein a similar deletion of paternal origin results in Prader-Willi syndrome (PWS) [Knoll et al., 1989; Magenis et al., 1990; Nicholls, 1993; Zackowski et al., 1993]. Thus, AS arises exclusively from the lack of expression of a maternally expressed gene(s), whereas PWS arises from loss of expression of a paternally expressed gene(s). Paternal uniparental disomy of chromosome 15 accounts for only 3–4% of instances of AS [Nicholls, 1993]. Imprinting mutations ($\leqslant$5% of AS patients; Driscoll, unpublished data) may also effectively result in the absence of a functional maternal copy of the region [Glenn et al., 1993; Reis et al., 1994; Buiting et al., 1995; Saitoh et al, 1996b].

Most examples of AS are sporadic. However, there have been instances of familial AS, most often involving sibs in whom no chromosome 15 abnormality was found [Meijers-Heijboer et al., 1993; Wagstaff et al., 1993], or in whom an imprinting mutation was demonstrated [Buiting et al., 1995; Saitoh et al., 1996a,b]. Few deletion-positive recurrences have been reported. These rare familial cases of deletion-positive AS may arise secondary to parental translocations [Hultén et al., 1991; Smeets et al., 1992; Freeman et al., 1993; Surh et al., 1994], a heritable deletion [Saitoh et al., 1992], or a heritable inversion [Clayton-Smith et al., 1993] of chromosome 15. In contrast, the families reported here have AS recurrences inexplicable by previously accepted mechanisms. Molecular cytogenetic analysis demonstrated a typical maternal deletion in each patient, each arising presumably by coincidence.

Contract grant sponsor: NIH; Contract grant number: HD31491.

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Received 23 May 1996; Accepted 3 September 1996

MATERIALS AND METHODS

Clinical Diagnosis

Clinical diagnosis was recently confirmed by the same geneticist in patients 1 and 2, and originally made by two different geneticists in patients 3 and 4. Clinical diagnosis was based on typical developmental histories, and morphologic, behavioral, and neurological characteristics [Williams et al., 1995a].

Cytogenetic Analysis

Peripheral lymphocytes were cultured and prepared for cytogenetic assessment using standard methods. G-banded preparations had band levels of 650 and 625 (patient 1), 800 and 625 (patient 2), 600 (patient 3), and 800 (patient 4) bands. Patients 3 and 4 have been previously referred to as AS124 and AS133, respectively [Driscoll et al., 1992; Zackowski et al., 1993; Glenn et al., 1996].

Fluorescent in situ hybridization (FISH) was completed on patients 1 and 2 using the following probes (Oncor, Inc., Gaithersburg, MD): Prader-Willi A (*D15S11*), Prader-Willi B (*GABRB3*), and *D15S10* (patient 1); and *SNRPN* and *D15S10* (patient 2). Parents of patients 1 and 2 were also assessed using the *D15S10* probe.

Molecular Analysis

Southern blot analysis of DNA samples was used to investigate the parent-of-origin DNA methylation imprint at the *SNRPN* first exon/promoter in all four patients, and also at *D15S9* (DN34) in patients 3 and 4 [Driscoll et al., 1992; Glenn et al., 1996]. Patients 3 and 4 were also evaluated for extent of deletion using loci and techniques described in Nicholls et al. [1989].

CLINICAL REPORTS

Family A

In this family first-cousins-once-removed (patient 1 and 2) were diagnosed with AS (Fig. 1).

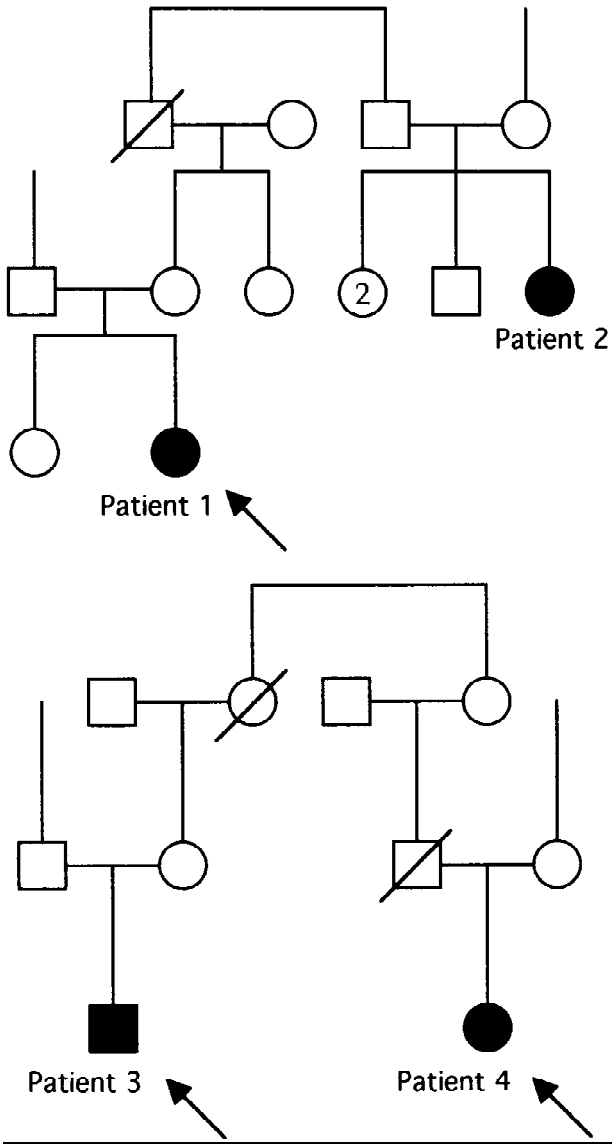

Fig. 1. Pedigrees of family A (**top**) and family B [previously described in Hendrickson et al., 1992] (**bottom**). Darkened symbols represent individuals affected with AS.

The proposita (Fig. 2), born in 1991, is the second daughter of nonconsanguineous parents and has a healthy sister. She was born at term, with birth weight of 3.6 kg (75th centile) and birth length of 51.3 cm (~80th centile). She had initial feeding problems which improved after 3 or 4 days. Concerns arose at around age 2 months because she neither fixed nor tracked. By 4 months it was recognized that she could not lift or control her head and by 6–7 months her parents were concerned because she could not sit. Likewise at about this time, episodes when her body would "freeze" (probably first onset of seizures) arose. At age 9 months she was assigned a tentative diagnosis of cerebral palsy.

The patient is moderately mentally retarded (estimated developmental quotient of 49 using the Vineland Adaptive behavior scales). She has never used any words but does sign "more" and "eat." Her favorite mode of transportation is to seat-scoot. She also walks with a walker and is transported in a wheelchair for long distances. She does some self-feeding. No loss of skills has been recognized. She has some unusual behaviors that seem consistent with the diagnosis of AS including periodic inappropriate laughter, although she does cry when she is in pain, and exhibits considerable arm flapping when she is excited. She has had occasional difficulties with circadian cycling including going as long as 2 full days without any sleep. She is obsessed with water.

Overt seizures had their onset at about age 2 years and are currently well controlled with divalproex sodium and phenobarbital. Electroencephalography at 2 years showed generalized sharp wave discharges and spike and slow wave discharges more prominent over the frontal regions bilaterally.

Clinical examination at almost 4 years shows the following: height 99.0 cm (~25th centile), weight 17.5 kg (~75th centile), and OFC 46.2 cm (~–3 SD). Facial

Fig. 2. Family A: Patient 1 (**A,B**) at age 4 years, and patient 2 (**C**-**E**) at age 16 years (C) and age 36 years (D and E).

features are pretty but consistent with AS, including mildly downslanting palpebral fissures, epicanthic folds, broad nasal base and nose, protuberant tongue, prominent lower vermilion, narrowed anterior palate, and a V-shaped mandible, which she often holds in a jutted out position. She has mild prominence of venous patterning over the chest and a minimal pectus excavatum. There is mild kyphosis at the dorsolumbar junction. She has hyperextensibility of the metacarpal-phalangeal joints and tends to hold her proximal interphalangeal joints in flexion. The left hand has a single palmar crease. Arms and legs are hypertonic but strong. She everts out over her ankles and has rather marked pes planus. She can dorsiflex her ankles beyond neutral and has heel strike. Deep tendon reflexes are normal and symmetric. She has intermittent ataxic and choreiform-like movements. When standing, she has a markedly broad base, and her gait is very ataxic.

All findings are consistent with a diagnosis of AS, although she is considerably more engaging and receptive than many individuals with Angelman syndrome. She also appears to have somewhat better receptive language abilities than anticipated.

Patient 2 (Fig. 2), born in 1959, is the first daughter of nonconsanguineous parents, with a healthy younger sister. The patient was born at term after a difficult labor in which forceps were used for delivery. There was a nuchal cord but her color was good, she had a good cry, and there were no apparent neonatal sequelae. Birth weight was 3.0 kg (25th centile) and birth length was 51.3 cm (~80th centile). Hospital reports state she was slightly hypertonic and subject to regurgitation of food.

Developmental problems were of considerable concern early in life and resulted in institutional placement at age 4 years, at which time she could seat-scoot and was learning to grasp items that were handed to her but could not walk or crawl, did not talk, was not toilet trained, and did not play with toys. All subsequent assessments of her developmental capabilities have demonstrated profound mental retardation, although she was also reported to be a "happy, social child." In addition, an evaluation in 1989 reported that she "may also laugh and cry for no apparent reason." Currently she follows simple commands, eats independently, needs assistance with drinking, requires help dressing, is able to stand, walks with minor assistance, can manipulate her wheelchair, is partially toilet trained, and has an intermittent, severe sleep disturbance. Psychological evaluation suggests an overall cognitive age of about 14 months.

In addition to her profound developmental retardation, patient 2 has a history of seizures, with age of onset of 1 year, and which have continued. Currently her seizures are well controlled with ethosuximide. Early electroencephalography showed generalized slowing and possibly spike and slow wave discharges, while more recent electroencephalography (in 1980 and 1990) showed synchronous spikes, and spike and slow wave pattern with the latter mostly in a bifrontal distribution.

Angelman syndrome was first suggested as a diagnosis in 1985, at age 26 years. Clinical examination at age 36 y shows the following characteristics: height 157.1 cm (~10th centile), weight 47 kg (10th centile), and OFC 50.4 cm (<2 SD below the mean). She has mild to moderate plagiocephaly with particular flattening of the right occipital region. There is marked diminution of vertical dimension of the lower face. She has a persistent exotropia and marked photophobia. Nasal base is prominent, the nasal tip is somewhat bulbous, the nasal septum is asymmetric, and the alae nasi are horizontally flared. Philtrum is very short. The mouth is exceedingly wide, and she has marked eversion and prominence of the lower lip. The mandible is short vertically but prominent. Arms show marked limitation of movement of all joints. She has long, narrow, and hypermobile thumbs. She has contractures and positional abnormalities of both feet, with the right side more severe. Heel cords are exceedingly tight and dorsiflexion is to less than neutral on the right. Skin shows patchy hypopigmentation and multiple scars, and is thin. Tone is asymmetric and moderately increased throughout with slow relaxation. Deep tendon reflexes are exceedingly brisk and asymmetric as well. Response to Babinski stimulation is mixed on the right and clearly upgoing on the left. She had a paroxysm of explosive vocalization once during examination. Her most medically troublesome problem is systemic lupus erythematosus (which we assume is unrelated to AS and which may account for her joint limitations).

Family B

In this family, second cousins (patients 3 and 4) have AS (Fig. 1).

Patient 3 (Fig. 3) was born in 1984, the only child of nonconsanguineous parents. He was delivered by Cesarean section because of breech presentation. Birth weight was 3.71 kg (~80th centile) and length was 52.6 cm (70th centile). As a newborn infant, he required oxygen for his first half day of life. Around 7 months he was noted to have flailing arm movements. Psychomotor development was delayed. The patient developed seizures at about age 1 year and has received anticonvulsant medications since then. Electroencephalography has shown generalized 2–3 Hertz polyspike and wave activity, mostly in the posterior regions, with generalized slowing.

Clinical examination in 1988 at age 4 years showed an active child with jerky movements. Height was 101.2 cm (40th centile), weight was 14.3 kg (15th centile), and OFC was 47.3 cm (<2 SD below the mean). He did not have significant occipital flattening. Facial appearance was reminiscent of AS in that he had a large mouth, prominent lower vermilion, protruding tongue, and moderate prognathism. Hair, eyelashes, and irides were very lightly pigmented. His limbs showed generalized hyperextensibility. He had mild hypertonia and his reflexes were very brisk throughout. He walked with a jerky gait and tended to hold his arms upward. He was unable to speak and on occasion became very excited, with flailing arms and legs.

Currently, although he has no speech, the patient communicates desires to his parents through gestures and eye movements. He began walking at age 7 years. He remains active and is capable of eating and drinking independently.

Patient 4 (Fig. 3) was born in 1968. During the pregnancy, her mother underwent surgery for a vaginal cyst, and an amniotic fluid leak occurred due to a fall at 7 months gestation. Birth weight was 3.17 kg (~45th centile) and length was 51.3 cm (65th centile). Excess secretions at birth led to hypoxia, and she was placed in an isolette with oxygen for a few days. Early development was delayed, and she did not say her first word until age 9 years. She has had a petit mal seizure disorder since 1 1/2 years of age. Seizures have been difficult to control. Electroencephalography was markedly abnormal and compatible with the seizure disorder with some diffuse slowing and prominent spikes.

On clinical examination in 1980 at age of 11 1/2 years, she manifested paroxysms of laughter and frequent tongue protrusion. Height was 121 cm (<5th cen-

Fig. 3. Family B: Patient 3 (Reproduced from Williams et al., 1995b, with permission from Mosby Publishing) (**A**) at age 7 years and patient 4 (**B**) at age 11 years.

tile) and weight was 46 kg (5th centile). She exhibited a moderate degree of prognathism and apparently hypopigmented eyebrows, eye lashes, and irides. She also had marked lordosis, a slight thoracic scoliosis, and foot eversion. She had generalized hypotonia and weakness. Her gait was "robot-like" with marked ataxia. She slapped her feet when walking, with lack of arm coordination.

At the time of last contact, "mama" was the patient's only word, and she demonstrated little understanding of verbal commands. She remained ataxic with absent speech and fewer episodes of excessive laughter [Hendrickson et al., 1992].

RESULTS

G-banded chromosomes at the 600-800 band level in all four patients identified a typical chromosome deletion [data not shown; patient 3 in Zackowski et al., 1993]. These interpretations were confirmed in patients 1 and 2 by FISH by using Oncor probes specific for 15q11-q13 (data not shown). Patients 3 and 4 were shown to be deleted at five loci within 15q11-q13 from ML34 to IR10. Cytogenetic assessment (all families) and FISH analysis (Family A) of the parents were normal, indicating that each affected AS proband has a de novo deletion.

To determine the parental origin of the deletion in each case, DNA methylation analysis using a 5′ *SNRPN* probe [Glenn et al., 1996] was performed on all four patients (Fig. 4). *Not*I is a methylation-sensitive enzyme that does not digest DNA if the recognition site is methylated at a CpG dinucleotide, whereas *Xba*I is not sensitive to methylation. When DNA from normal individuals (lanes 2, 3, 7–9, and 13; Fig. 4) is digested with *Xba*I/*Not*I, a methylated, maternal band of 4.3 kb and an unmethylated, paternal band of 0.9 kb are produced. Control AS (lanes 4, 5, and 11), and PWS (lanes 6 and 12) deletion patients have only the lower or upper band, respectively. As expected, the AS patients 1 and 2 have only the lower, paternal band, indicating a maternal deletion (Fig. 4). Likewise, maternal origin of the deletion for patients 3 and 4 were identified using DNA methylation at *SNRPN* [Glenn et al., 1996; data not shown] and at *D15S9* (DN34) [Driscoll et al., 1992; data not shown].

DISCUSSION

Upon first glance, these families appeared to be examples of a new mode of transmission in AS and inexplicable by current theory. They represent an unexpected recurrence for several reasons.

First, in each case, the father of one affected individual is the relative linking the other affected individual, which seems to contradict the accepted maternal origin of AS deletions. Indeed, there has been no documented instance of paternal transmission of a deletion resulting in a child affected with AS. Therefore, an inherited cause for the absence of the appropriate portion of chromosome 15 would be expected to result in the child of the linking male being diagnosed with PWS, not AS. Saitoh et al. [1992] reported on a family in which a submicroscopic 15q11-12 deletion was

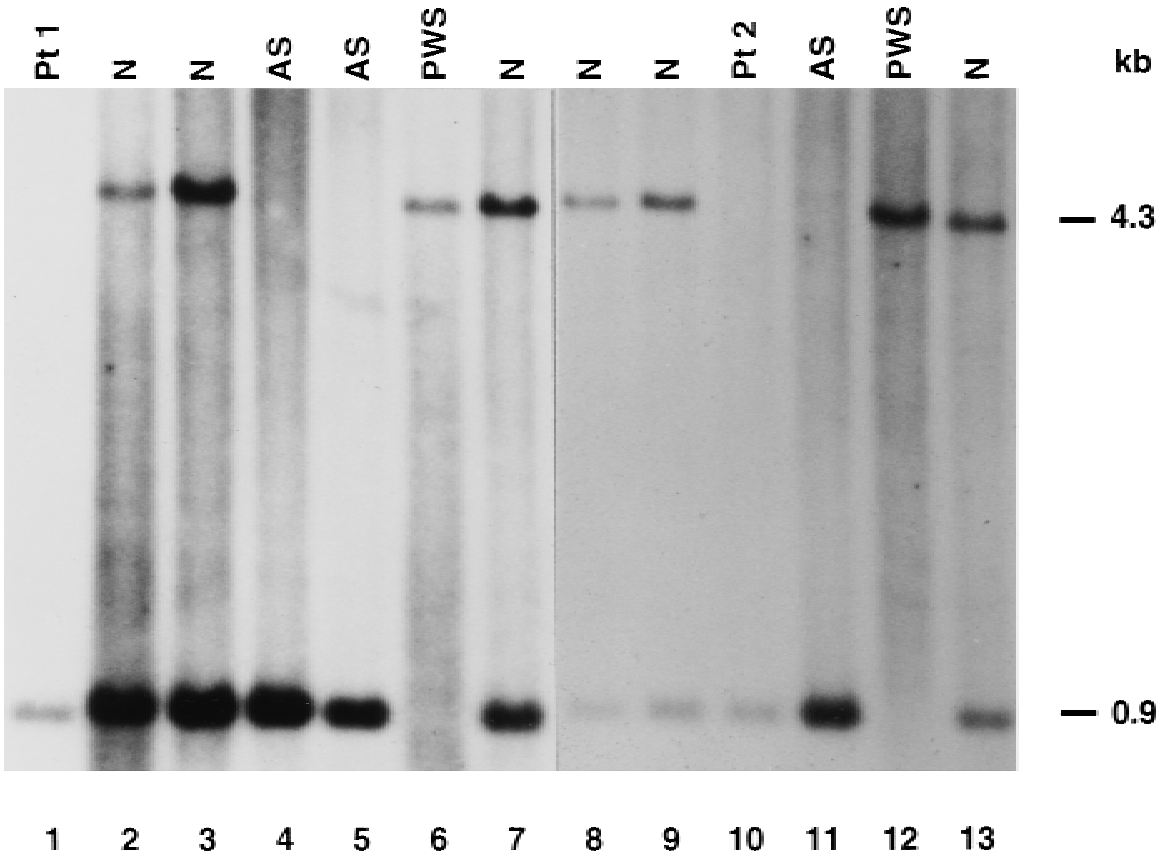

Fig. 4. DNA methylation analysis of Family A. DNA from peripheral blood leukocytes were digested with *Xba*I/*Not*I and a Southern blot was hybridized with a 0.6 kb *SNRPN* probe. Normal individuals (N, **lanes 2, 3, 7–9**,and **13**), control AS (**lanes 4, 5,** and **11**), and control PWS (**lanes 6** and **12**) deletion patients, and patients 1 (Pt. 1) and 2 (Pt. 2) are shown.

passed from father to daughter with no phenotypic expression of PWS in the daughter, although she then had three children with AS, each of whom carries the deletion. However, this apparent exception is explicable because the microdeletion involved a segment including only the *D15S10* to *GABRB3* loci, which is not sufficient to cause the PWS phenotype. Nor would one expect an AS phenotype in this woman because the paternal contribution for this site is expected to be silent due to imprinting. Hall and Smith [1972] described maternal first cousins with PWS, related through the unexpected parent (i.e., the obverse of the families described here). However, in this family one patient was clinically atypical, and it is not known whether molecular cytogenetic follow-up has been performed.

Secondly, most familial recurrences in AS involve sibs without currently detectable genetic abnormalities [Meijers-Heijboer et al., 1993; Wagstaff et al., 1993] or with imprinting microdeletions [Buiting et al., 1995; Saitoh et al., 1996a,b]. The few recurrences that have involved deletion-positive patients can be explained by abnormalities in a parental chromosome (i.e., translocation or paracentric inversion) [Saitoh et al., 1992; Hultén et al., 1991; Smeets et al., 1992; Clayton-Smith et al., 1993; Freeman et al., 1993; Surh et al., 1994]. No such parental chromosomal abnormality is present in either family reported here. Recently, an inbred family with three recurrences of AS was described by Beuten et al. [1996]. However, one patient had UPD, whereas the other two patients had imprinting mutations of apparently independent origin.

Because the recurrences reported here seemed inexplicable by current models, further investigation was warranted. Cytogenetic analysis of the patients confirmed a typical 15q deletion in each patient, and molecular studies demonstrated a maternal origin of these deletions. Cytogenetic analysis of the parents found no chromosomal rearrangement such as a translocation or paracentric inversion that could predispose to deletions, confirming that these recurrences cannot be explained by previously described mechanisms. Finally, we verified that the mother-pairs in each family were not known to be related to each other.

It is conceivable that a shared environment contributed to the recurrence of AS reported here. The two mothers of the AS children in family A did live in the same town, although never at the same time. For this reason, cursory occupational and pregnancy exposure histories were obtained from these mothers, which produced no evidence of shared exposures; nor did local inquiries produce any recognized hazards in the shared town. In the absence of properly conducted epidemiological studies, it is unknown if environmental hazards can contribute to the risk of occurrence of such deletions. However, preliminary studies on the similar de-

letion in PWS suggested that there was an increased incidence of hydrocarbon exposure among fathers of children with PWS [Strakowski and Butler, 1987; Cassidy et al., 1989], suggesting further studies on exposure risks in PWS and AS are warranted. If this were to be studied further, pregnancy exposures of the maternal grandmothers of the AS patients should be investigated as well.

Can these recurrences be attributed solely to chance occurrence of independent events? To estimate the frequency with which pedigrees with two individuals with the same disorder would arise secondary to two independent mutational events (i.e., by chance), [Reiser et al., 1984] estimates of the mutation rate for the disorder in question must be available. The population frequency of AS has been estimated as approximately 1/20,000 (5×10^{-5}) [Clayton-Smith and Pembrey, 1992]. If one assumes equal survival among AS patients and the general population, then the population frequency can be used as a placeholder for the mutation rate. Alternatively, one can generate independent estimates of the population frequency of AS by comparing in a specific population the number of AS diagnoses with the number of diagnoses of some other disorder for which better established population frequencies are available. While such calculations require simplifying assumptions and may include ascertainment biases, if multiple such comparisons yield similar estimates, then those estimates are likely to be reasonable approximations of the true frequency of AS. For this purpose, the Clinical Genetics Center, University of Wisconsin-Madison clinical database was searched for patients diagnosed from 1988 to the present with Angelman syndrome, Prader Willi syndrome, and Down syndrome. In addition, cytogenetic laboratory databases from the Waisman Center Cytogenetics Laboratory, Madison, WI, and the Wisconsin State Laboratory of Hygiene-Cytogenetics Laboratory, Madison, WI were searched for patients (1992–present) who were confirmed (by FISH) to carry a chromosome 15 deletion and who were diagnosed as having either AS or PWS since 1992. These data were then used to develop a range of estimated frequencies of Angelman syndrome (Table I). All estimates range from about 1/20,000 to about 1/30,000, confirming that use of the previously published frequency estimate (and therefore mutation rate) of 5×10^{-5} [Clayton-Smith and Pembrey, 1992] is reasonable.

Second, an estimate of how many relatives are ascertained through a particular patient is needed. Review of family histories of new propositi with AS or PWS assessed over the last 4 years at the University of Wisconsin Clinical Genetics Center shows that, on average, information was obtained on about 64 additional relatives (not including parents) (n = 17, mean = 63.7, SD = 39.6). We assume that this number is typical of families with an AS index case seen in other genetics clinics as well.

Using an estimated mutation rate for AS of 5×10^{-5} yields an estimate of the number of cases of AS in North America of about 14,400 (total population of U.S. and Canada × mutation rate; or 288,828,000 [Famighetti, 1994] $\times 5 \times 10^{-5}$). With 64 relatives ascertained by family history and assuming complete ascertainment of AS cases within these relatives, 46 familial recurrences could be anticipated to have arisen *by chance* [total number of AS cases × mutation rate × number of identified relatives of index case; $14{,}400 \times (5 \times 10^{-5}) \times 64$]. Therefore, the null hypothesis that the recurrences reported here arose simply by chance can not be rejected. (Even if one chose to use the lowest estimates in Table I, this conclusion would not change.) However, one might argue that it is no more likely for a familial recurrence to be identified and reported than for Angelman syndrome in general. If familial cases are not preferentially reported or identified, then, given that only approximately 500 cases of AS are known to the Angelman Syndrome Foundation [Williams et al., 1995b], that figure could be used to find a *minimal* number of familial recurrences that would be expected to have arisen by chance: $500 \times (5 \times 10^{-5}) \times 64$, or ≈2. Even if the family of Beuten et al. [1996] is a third example of such unexpected recurrence (involving a different molecular abnormality), once again, even with these minimizing assumptions, the null hypothesis cannot be rejected.

Genetic disorders can be expected to recur within families on occasion without those recurrences necessarily having any fundamental biological significance. The possibility of chance events must be kept in mind when using such rare events to postulate novel mechanisms.

Certainly, conclusions would be more straightforward if more precise estimates of the frequency of AS were available. As for many other genetic disorders, population based ascertainment of the frequency of AS is a vital need.

TABLE I. Estimation of Population Frequency and Mutation Rate in Angelman Syndrome*

Source of estimate	Comparison diagnosis	Ratio of Angelman/ comparison diagnosis	Population frequency of comparison diagnosis	Derived estimated frequency of Angelman syndrome
Clayton-Smith and Pembrey, 1992	—	n.a.	n.a.	5.0×10^{-5}
UW Clinical Genetics Center	Down syndrome	10/188	1.0×10^{-3}[a]	5.3×10^{-5}
UW Clinical Genetics Center	Prader-Willi syndrome	10/16	6.2×10^{-5}[b]	3.9×10^{-5}
Wisconsin Cytogenetics	Prader-Willi syndrome	4/8	6.2×10^{-5}[b]	3.1×10^{-5}

*In each instance estimated relative frequency for AS was calculated by comparison to estimates of population frequencies of Down syndrome and PWS, which represent disorders with reasonably well-established population frequencies of high and low magnitude, respectively. Ratios of Angelman/ Comparison Diagnosis are raw numbers generated as described in the text. n.a., not applicable.
[a]Serra and Neri, 1990.
[b]Burd et al., 1990.

ACKNOWLEDGMENTS

The authors thank Harinderjeet Singh B.S., Gurbax S. Sekhon Ph.D., Catherine G. Palmer Ph.D., Gail H. Vance M.D., William Adkins M.D., Elizabeth Pendleton I.C.S.W., Susan Kirkpatrick M.S., Lorraine Meisner Ph.D., Eric B. Johnson B.S., and Kim Horton R.N., M.S. for their assistance collecting data, and the families for their gracious cooperation. This work was supported in part by NIH grant HD31491 (to R.D.N. and D.J.D.).

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